REMARKS

Please cancel claims 11-25, 27 & 32-35 12/11/01 JS

The claims in the case are 1-10, and 28, and 30-31. Applicants note, that claims 11-25, 27 and 32-35 are withdrawn from consideration. Applicants herein request cancellation of Claims 9, 11-27, 29, and 32-35, without prejudice, and hereby reserve the right to prosecute these claims in one or more divisional application. After this Amendment, the claims pending are 1-8, 10, 28, 30, and 31.

Applicants further note, with appreciation, allowance of Claims 2 and 3. Minor changes have been introduced into these claims in order to claim nucleic acid "molecules" rather than "sequences".

Applicants submit herewith an amended Table II (attached as Appendix A), as helpfully suggested by the Examiner, and request that the amended Table II, which corrects spacing problems, be entered in place of Table II as filed. No new matter is added.

Applicants request that the Sequence Listing, submitted on May 8, 2000, be entered before the claims, which begin on page 43 of the Specification as filed. Furthermore, Applicants have amended the Specification to include reference by sequence identifiers to all the sequences shown in Figures 1 and 2. The legend for Figure 1 was amended on January 12, 2001, and the legend for Figure 2 is amended herein. Note that the sequences in Figure 2 correspond to the sequence identifiers in the Sequence Listing. Thus, no new matter is added. Applicants respectfully submit that the Application complies with the directives of 37 CFR §§1.821 (a)(1) and (a)(2).

Applicants respectfully submit that the priority data is now correctly referenced for the Application and hereby submit a new Declaration referencing the same.

Applicants earnestly submit that no new matter is introduced by the amendment submitted for page 34, lines 9 and page 35, lines 9 and 32, in the response filed on January 12, 2001. Applicants believe that the Examiner is referring to the amendment on page 35, line 32, rather than line 13, as no amendment was requested for page 35, line 13. Regarding the amendment on page 34, line 9, the original disclosure supports the single human EST and its reference number. Applicants invite the Examiner's attention to page 33, lines 25-26 wherein it is states that one EST was found that was homologous to the murine TRELL sequence. This clone has the GenBank Accession number R33579.

We have requested cancellation of the matter added in the previous amendment at page 35, line 9. We have further requested amending this section to end the sentence after the words "sizing analysis" so that the sentence makes sense. Note that the sentence simply states that "Purified TRELL was subjected to sizing analysis." The sentence now does not specify how this was performed, but nevertheless, does not add new subject matter.

Regarding the amendment on page 35, line 32, without conceding the correctness of the Examiner's position, we hereby request cancellation of the subject matter added in the previous amendment filed on January 14, 2001.

The Applicants have amended claim 1 to remove the word "equivalent." Applicants have also amended claim 10, as helpfully suggested by the Examiner to clarify the steps of obtaining

substantially purified TRELL. We respectfully request withdrawal of the rejection under 35 U.S.C. § 112, second paragraph.

Regarding the new matter rejection in connection with claim 4, the claim has been amended to recite that the nucleic acid sequence encodes a protein that is at least 50% homologous with the receptor binding domain of TRELL. The specification describes Tumor Necrosis Factor Related Ligands as part of the TNF family of proteins having a C-terminal extracellular region that is the receptor binding portion of the protein. As stated in the Specification at page 7, lines 15 to 17, the DNA sequences include those that are at least 50% homologous with the C-terminal portion of Tumor Necrosis Factor Related Ligand and hybridize with the claimed sequences and fragments thereof.

35 U.S.C. § 112, First Paragraph

Turning to the rejections under 35 U.S.C. § 112, first paragraph, Applicants hereby request cancellation of claims 9, 26 and 29 without prejudice and without disclaimer as to the subject matter thereof. Applicants further reserve the right to pursue the subject matter of these claims in one or more divisional applications. The rejection of these claims is now moot.

Applicants have amended claim 1 to claim isolated nucleotide molecules having sequences that encode the amino acid sequences comprising SEQ ID NO:2 or SEQ ID NO:4. It is respectfully submitted that "isolated" would be understood by one of ordinary skill in the art to exclude genomic sequences in their natural state. On page 10, lines 12-14, the Specification describes nucleic acid sequences encoding TRELL that are "substantially free of a nucleic acid sequence with which it

occurs in the organism from which the nucleic acid is derived." Thus, the Specification provides support for the term "isolated" although not *in haec verba.*

Claim 4 has been amended to specifically claim the percent homology described in the Specification at page 7, lines 15-18, and to include the specific hybridization conditions set forth in the specification at pages 30 and 34. We respectfully submit that claim 4 is in condition for allowance.

Applicants have amended claim 5 to recite TRELL sequences having conservative amino acid substitutions, alterations and deletions that do not affect the biological activity of TRELL. It is taught in the specification that the active site of TRELL is a weak inducer of apoptosis in cell assays (see Tables II and III). One of ordinary skill in the art will be able to make substitutions, alterations and deletions using the cytotoxicity assay provided in the specification by routine experimentation to determine whether the TRELL protein containing the substitution, alteration or deletion affect the biological activity of TRELL. The person of ordinary skill in the art is provided with ample structural information as the nucleic acid molecules must encode the TRELL amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4 containing only conservative amino acid changes. We earnestly submit that one of ordinary skill in the art is given sufficient guidance with respect to the polynucleotide variants such that it is apparent that the applicants were in possession of the invention at the time of filing.

Claim 6 has been amended to recite that the nucleotide sequence encoding TRELL (SEQ ID NO:2 or SEQ ID NO:4) is operably linked to an expression control sequence (through reference to

claim 1). Such subject matter is specifically supported at page 12, lines 3-9, for example. Therefore, no new matter is added. As claim 1 has been amended to overcome the outstanding objection, it is submitted that claim 6 also overcomes the outstanding objection.

Applicants have amended claim 7 to specifically recite that the nucleic acid molecule of claim 6 comprises SEQ ID NO:1 or SEQ ID NO:3. As claim 1 has been amended to overcome the outstanding objection, it is submitted that claim 7 also overcomes the outstanding objection.

Claim 8 has been amended to specifically recite a "host cell" rather than simply "host" comprising the nucleic acid molecule of claim 6 or 7. Applicants submit that the clarification does not alter the scope of the claim.

Claim 10 has been amended, as helpfully suggested by the Examiner, and further through the amendment of the claims from which it depends. Applicants submit that claim 10 is in condition for allowance.

Claim 26 has been cancelled, therefore, the rejection is moot with respect to this claim.

Claim 28 has been amended to recite that the nucleic acid sequence encoding TRELL comprises the amino acd sequence set forth in SEQ ID NOs:2 or 4.

Claim 29 has been cancelled, therefore, the rejection is moot with respect to this claim.

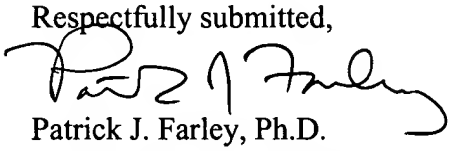
Claims 30-31 have been amended to depend from claim 28. Applicants submit that the claims, as amended, are in condition for allowance.

35 U.S.C. §102(b)

Claims 1, 4, 5 and 26 have been previously rejected under 35 U.S.C. § 102(b) over Matsubara. The present office action does not specifically withdraw the rejection. Claim 1 has been amended to specifically recite a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:3. The nucleic acid sequence disclosed in Matsubara that has 97.3% local similarity to SEQ ID NO:3 does not encode any portion of the TRELL protein, let alone the receptor binding domain of TRELL. Thus, claim 4, which now specifically recites that the purified DNA molecule encodes a protein that is at least 50% homologous to the receptor binding domain TRELL, is not anticipated by Matsubara. Likewise, the sequence shown in Matsubara to be 97.3% similar does not encode a TRELL protein. Thus, claim 5, which recites that the nucleic acid sequence encodes TRELL, with conservative amino acid substitutions, alterations or deletions that do not abolish the biological activity of TRELL, is not anticipated by Matsubara. Claim 26 is cancelled herein, rendering the rejection with respect to this claim moot. We earnestly submit that the claims are patentable over Matsubara.

We earnestly submit that the claims are condition for allowance, which action is respectfully requested.

Respectfully submitted,



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Version with Markings to Show Changes Made

IN THE SPECIFICATION

RELATED APPLICATIONS

The present application is a continuation of International Application PCT/US97/13945, filed August 7, 1997, which claims [priority] benefit under 35 U.S.C. §119(e) to U.S. Application Nos. 60/023,541, filed August 7, 1996; 60/028,515, filed October 18, 1996; and 60/040,820, filed March 18, 1997, all now expired.

Figures 2A and 2B are an amino acid comparison of human members of the TNF family including: hTNF (SEQ ID NO:19); hLT-α (SEQ ID NO:20); hLT-β (SEQ ID NO:21); hFasL (SEQ ID NO:22); hTFRP (SEQ ID NO:4); hTRAIL (SEQ ID NO:23); hcD27L (SEQ ID NO:24); hCD30L (SEQ ID NO:25); hCD40L (SEQ ID NO:26); h4-1BBL (SEQ ID NO:27).

Analysis of Secretion

Vectors for EBNA based expression were constructed using the vector CH269 which is a modified version of the pEBVHis ABC (Invitrogen) wherein the EBNA gene and the histidine tag were removed. A 0.71 kb fragment of hTNF in the pFastBac vector was provided by Dr. P. Pescamento and A. Goldfeld. The SnaBI/XhoI insert was ligated into the PvuII/hoI site of CH269. A genomic TNF insert containing the _1-12 cleavage site deletion was a gift from Dr. G.

Kollias and was inserted into the CH269 vector by A. Goldfeld. A 1.8 kb NotI insert of hTRELL clone A2A, the 0. 98 kb NotI fragment containing the hCD40L cDNA provided by Dr. E. Garber and a 1.46 kb NotI insert containing hLTa (Browning et al., 1995) were ligated into the NotI site of CH269. A 0.81 kb HindIII insert containing the hLTb coding region with a modified start site (Browning et al., 1995) was ligated into the HindIII site of CH269. EBNA-293 cells were transfected with the various CH269 vectors along with the GFP vector using lipofectamine and either removed with PBS with 5 mM EDTA for FACS analysis or after 2 days the cells were subjected to metabolic labelling. Both procedures utililized the following antibodies, hTRELL a rabbit polyclonal Ig fraction, hTNF the mAb 104c, hLTa the mAb AG9, LTal/b2 the mAb B9 and CD40L the mAb 5C8. FACS analysis was carried out in RPMI medium containing 10% FBS and 50 ug/ml heat aggregated human IgG with the antibodies at 5 ug/ml. Phycoerythrin labelled anti-mouse or rabbit IgG (Jackson ImmunoResearch) was used to detect antibody binding. GFP bright transfected cells were live gated. For immunoprecipitation, cells 2 days after transfection were washed with PBS and transfered into met/cys free MEM containing 200 uCi/ml TranSlabel (ICN). After [3]__ h the supernatants were harvested and subjected to immunoprecipitation as described (Browning et al., 1995).

IN THE CLAIMS

1. (Twice Amended) [A substantially purified DNA sequence] An isolated nucleic acid molecule encoding TRELL, wherein said [DNA] TRELL comprises the amino acid sequence [comprising:

(a)] SEQ ID [NO:1] NO:2 or [an equivalent thereof;

(b)] SEQ ID [NO:3 or an equivalent thereof] NO:4.

2. (Allowed: Twice Amended) A substantially purified DNA [sequence] molecule encoding TRELL, said sequence consisting essentially of SEQ ID NO:1 or SEQ ID NO:3.

3. (Allowed: Twice Amended) A substantially purified DNA [sequence] molecule consisting essentially of SEQ ID NO:1 or SEQ ID NO:3, said DNA encoding a polypeptide, said polypeptide consisting essentially of SEQ ID NO:2 or SEQ ID NO:4.

4. (Twice Amended) A substantially purified DNA [sequence] molecule that hybridizes under stringent conditions to at least a fragment of SEQ ID NO:1 or SEQ ID NO:3, said fragment comprising at least 20 consecutive bases, said DNA sequence encoding a polypeptide that is at least [30%] 50% homologous with the receptor binding domain of TRELL, wherein said stringent conditions comprise washing steps using 2X SSC, 0.1% SDS at 65°C.

5. (Twice Amended) A substantially purified DNA [sequence] molecule wherein said [sequence] molecule encodes TRELL, wherein said TRELL comprises the amino acid sequence SEQ ID [NO:1] NO:2 or SEQ ID [NO:3] NO:4, wherein said amino acid sequence comprises [with] conservative substitutions, alterations or deletions which do not abolish the biological activity of TRELL.

6. (Amended) [A recombinant DNA] The nucleic acid molecule of claim 1 [comprising a DNA sequence encoding TRELL, said sequence] operably linked to an expression control sequence.

7. (Twice Amended) The nucleic acid molecule of claim 6 comprising SEQ ID NO:1 or SEQ ID NO:3.

8. (Amended) A host cell transformed with [a recombinant DNA] the nucleic acid molecule of claim 6 or 7.

10. (Twice Amended) A method for producing substantially pure TRELL comprising the steps of culturing the host cell of claim 8 and [substantially purifying] isolating TRELL from said transformed host cell to obtain substantially purified TRELL.

28. (Amended) A method of expressing [a gene] TRELL in a mammalian cell comprising:

a. introducing [a gene] a vector comprising a nucleic acid molecule comprising a sequence encoding TRELL into a mammalian cell, wherein said TRELL comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;

b. allowing said cell to live under conditions [that said gene] wherein said nucleic acid molecule is expressed in said [mammal] mammalian cell.

30. (Amended) The method of claim [29] 28 wherein [the mammal] said mammalian cell is a human cell.

31. (Amended) The method of claim [29] 28 wherein said vector is a virus.

Appendix A

Table II: Human TRELL Binding Sites and Cytotoxic Effects on Various Cell Lines

Cell Line	Type	TRELL Binding	Cytotoxicity[a]
Hematopoietic			
Jurkat	T lymphoma	-	-
SKW 6.4	EBV B cell		-
Namalwa	Burkitt lymphoma	-	-
K562	promyelocytic	+	-
THP-1	monocytic leukemia	++	-
Nonhematopoietic			
HT29	colon adenocarcinoma	+	++[b]
ME-180	cervical carcinoma		-
Hela	cervical carcinoma		-[d]
MCF-7	breast adenocarcinoma		+/-
293	embyronic kidney cells	+	ND[c]
Cos	kidney fibroblasts	+	ND

"-" = no binding/cytotoxicity; "+" = some binding/cytotoxicity; and "++" = significant binding/cytotoxicity

[a]3-5 day proliferation assay in the presence and absence of human interferon-g.
[b]Cytotoxicity was only observed in the presence of interferon-g.
[c]ND, not determined.
[d]Morphology changes